Supplemental Material to:

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Materials and methods

N^2,N^3-Bis-t-butoxycarbonyl-2,3-diaminopropanoic acid (**2**)

2,3-Diaminopropanoic acid monohydrochloride (**1**) (493.7 mg) and sodium bicarbonate (3.0697 g) were dissolved in water (25 ml) and dioxane (25 ml). To the solution was added di-*t*-butyl-dicarbonate (3.1352 g). The reaction mixture was stirred at room temperature for 8 h. The mixture was diluted with water (75 ml), washed with dichloromethane (2 × 30 ml), acidified to pH 2.5 with 5 N HCI, and extracted with dichloromethane (3 × 40 ml). The combined organic extracts were dried over sodium sulfate and concentrated under reduced pressure. The desired product (**2**) was obtained as white powder, yield 1.0591 g (98 %). ^{1}H NMR (DMSO, δ in ppm) 6.86 (d, 1H, J = 7.85 Hz, -N*H*CH-), 6.77 (s, 1H, -N*H*CH_2-), 3.97 (m, 1H, -C*H*CH_2-), 3,21 (m, 2H, -CHC*H*$_2$-), 1.36 (s, 9H, -C(C*H*$_3$)$_3$), 1.35(s, 9H, -C(C*H*$_3$)$_3$).

N^2,N^3-Bis-t-butoxycarbonyl-2,3-diaminopropanoic acid benzyl ester (**3**)

Compound **2** (556.7 mg), benzyl bromide (0.5 ml) and triethylamine (0.6 ml) were refluxed in THF (15 ml) overnight. The reaction mixture was filtered. The filtrate was concentrated, diluted with ethyl acetate (75 ml) and washed with 1 N HCl (100 ml), brine (100 ml), 1 N $NaHCO_3$ (100 ml) and brine (100 ml). The organic phase was dried over sodium sulfate, concentrated and purified by silica column chromatography (eluting with 3% to 5% methanol in chloroform) to give the desired product as white powder, yield 644.9 mg (89 %). ^{1}H NMR ($CDCl_3$, δ in ppm) 7.36-7.33(m, 5H, -Ar*H*), 5.45(s, 1H, -N*H*CH-), 5.17(s, 2H, ArC*H*$_2$-), 4.77(s, 1H, -N*H*CH_2-), 4.39(s, 1H, -C*H*CH_2-), 3.52(s, 2H, -CHC*H*$_2$-), 1.43(s, 9H, -C(C*H*$_3$)$_3$), 1.42(s, 9H, -C(C*H*$_3$)$_3$).

2,3-Diaminopropanoic acid benzyl ester bis-trifluroacetate (**4**)

The benzyl ester **3** (380.9 mg) was treated with a solution of trifluoroacetic acid (3.6 ml) and water (0.4 ml) at room temperature for 2 h. The reaction mixture was concentrated under reduced pressure to viscous oil, and diethyl ether (30 ml) was added. The precipitate was filtered and washed with diethyl ether to give white powder, yield 389.7 mg (95%). ^{1}H NMR (D_2O, δ in ppm) 7.40-7.35(m, 5H, -Ar*H*), 5.33(d, 1H, J = 11.97 Hz, ArC*H*$_2$-), 5.26(d, 1H, J = 11.97 Hz, ArC*H*$_2$-), 4.46(dd, 1H, J = 7.50 Hz, 5.79 Hz, -C*H*CH_2-), 3.56(dd, 1H, J = 13.88 Hz, 5.08 Hz, -CHC*H*$_2$-), 3.45(dd, 1H, J =13.88 Hz, 5.70 Hz, -CHC*H*$_2$-).

N^2,N^2,N^3,N^3-Tetrakis-(t-butoxycarbomethylene)-2,3-diaminopropionic acid benzyl ester (**5**)

Compound **4** (1.1495 g), *t*-butyl bromoacetate (2.9 ml) and DIEA (3.2 ml) were heated in DMF (6.5 ml) for 14 h at 70ºC. The reaction mixture was cooled to room temperature and filtered. The filtrate was concentrated under reduced pressure and diluted with EtOAc (20 ml) and hexane (80 ml). The solution was washed 1 N $NaHCO_3$ (2 × 50 ml). The organic phase was dried over sodium sulfate, concentrated and purified by silica column chromatography (eluting with 10 % EtOAc in hexane) to give the desired product as light yellow oil, yield 1.3109 g (74 %). ^{1}H NMR ($CDCl_3$, δ in ppm) 7.40-7.30(m, 5H, -Ar*H*), 5.15(d, 1H, J = 12.3 Hz, ArCH_2-), 5.10(d, 1H, J = 12.3 Hz, ArCH_2-), 3.66(dd, 1H, J = 5.35 Hz, 8.98 Hz, -C*H*CH_2-), 3.56(d, 4H, J = 5.38, -NCH_2CO-), 3.46(s, 4H, -NCH_2CO-), 3.15(dd, 1H, J = 13.55 Hz, 5.35 Hz, -CHCH_2-), 3.06(dd, 1H, J = 13.55 Hz, 9.15 Hz, -CHCH_2-), 1.42(s, 36H, -O(CH_3)$_3$).

N^2,N^2,N^3,N^3-Tetrakis-(t-butoxycarbomethylene)-2,3-diaminopropionic acid (**6**)

Compound **5** (1.3109 g) was hydrogenated under an atmosphere of hydrogen over 10 % Pd on carbon (145.7 mg) in methanol (20 ml) at room temperature for overnight. The reaction mixture was filtered, concentrated under reduced pressure to give desired product as light yellow gummy compound, yield 1.0123 g (90 %). ^{1}H NMR ($CDCl_3$, δ in ppm) 3.71(t, 1H, J = 6.95 Hz, -C*H*CH_2-), 3.57-3.45(m, 8H, -NCH_2CO-), 3.12(m, 2H, -CHCH_2-), 1.46(s, 36H, -O(CH_3)$_3$).

N^2-Fmoc-N^6-t-butoxycarbonyl-2,6-diaminohexanoic acid benzyl ester **(8**)

To the solution of N^2-Fmoc-N^6-*t*-butoxycarbonyl-2,6-diaminohexanoic acid (**7**) (6.00 g), DMAP (163.4 mg) and DIEA (3.4 ml) in dichloromethane (70 ml) were dropwised benzyl chloroformate (2 ml) in dichloromethane (20 ml) at 0ºC over 15 min. The reaction mixture was stirred at 0ºC for 3 h. The mixture was washed with 1 N $KHSO_4$ (2 × 100 ml) and brine (100 ml), dried over sodium sulfate and concentrated. The crude product was purified by silica column chromatography (eluting with 50 % ethyl acetate in hexane) to give the desired product as white powder, yield 7.12 g (99 %). ^{1}H NMR ($CDCl_3$, δ in ppm) 7.76(m, 2H, -Ar*H* Fmoc), 7.60(m, 2H, -Ar*H* Fmoc), 7.42-7.28(m, 9H, -Ar*H* Fmoc and Bn, overlapped), 5.40(s, 1H, -N*H*CH-), 5.21(d, 1H, J = 12.05 Hz, -CH_2Ar Bn), 5.15(d, 1H, J =

12.10 Hz, -CH_2Ar Bn), 4.51(s, 1H, -NHCH_2-), 4.41-4.37(m, 3H, -NHCH- and -CH_2CHAr Fmoc, overlapped), 4.22(m, 1H, -CH$_2$CHAr Fmoc), 3.08(s, 2H, -NHCH_2-), 1.87(m, 1H, -CHCH_2-), 1.72(m, 1H, -CHCH_2-), 1.58-1.31(m, 13H, -CHCH$_2$CH_2CH_2- and C(CH_3)$_3$, overlapped).

N^2-Fmoc-2,6-diaminohexanoic acid benzyl ester mono-hydrochloride (**9**)

The benzyl ester **8** (2.84 g) was treated with a solution of trifluoroacetic acid (50 ml) and dichloromethane (20 ml) at room temperature for 2 h. The mixture was concentrated under reduced pressure to viscous oil and treated with 1 M HCl in diethyl ether (50 ml). The precipitate was filtered and washed with diethyl ether to give the desired product as white powder, yield 2.52 g (99 %). ^{1}H NMR (DMSO, δ in ppm) 7.90(m, 2H, -ArH Fmoc), 7.70(m, 2H, -ArH Fmoc), 7.44-7.32(m, 9H, -ArH Fmoc and Bn, overlapped), 5.13(s, 2H, -CH_2Ar Bn), 4.35-4.22(m, 3H, -CH_2CHAr Fmoc), 4.06(s, 1H, NH$_2$CH-), 2.73(s, 2H, NH$_2$CH_2-), 1.72 (m, 1H, -CHCH_2-), 1.65(m, 1H, -CHCH_2-), 1.52(m, 2H, NH$_2$CH$_2$CH_2-), 1.34(m, 2H, -CHCH$_2$CH_2-).

N^2-Fmoc-N^6-EDTA-2,6-diaminohexanoic acid benzyl ester (**10**)

The carboxylic acid **6** (456.0 mg), HBTU (310.9 mg), HOBt (126.5 mg) and DIEA (450 µl) were dissolved in DMF (10 ml) and Pyridine (10 ml). The reaction mixture was stirred for 15 min. To the solution was added the amine (9) (451.4 mg) and stirred for overnight. The mixture was diluted with EtOAc (30 ml) and hexane (60 ml), washed with 1 N HCl (70 ml). The organic phase was dried over sodium sulfate, concentrated and purified by silica column chromatography (eluting with 2 % methanol in dichloromethane) to give the desired product as white powder, yield 438.6 mg (48 %). ^{1}H NMR (CDCl$_3$, δ in ppm) 8.42(m, 1H, -CONHCH$_2$-), 7.76(m, 2H, -ArH Fmoc), 7.60(m, 2H, -ArH Fmoc), 7.40-7.29(m, 9H, -ArH Fmoc and Bn, overlapped), 5.49(m, 1H, -CONHCH-), 5.20(d, 1H, J = 12.3 Hz, -CH_2Ar Bn), 5.16(d, 1H, J = 12.6 Hz, -CH_2Ar Bn), 4.41-4.22(m, 4H, -NHCH- and -CH_2CHAr Fmoc, overlapped), 3.54-3.36(m, 8H, -NCH_2CO-), 3.41(m, 2H, -NCH_2CH-), 3.18(m, 2H, -NHCH_2-), 2.89(m, 1H, -NCHCO-), 1.86(m, 1H, -NHCHCH_2-), 1.73(m, 1H, -NHCHCH_2-), 1.57-1.43(m, 40H, -CHCH_2CH_2CH_2- and -O(CH$_3$)$_3$, overlapped).

N^2,N^2,N^3,N^3-Tetrakis-(bismethoxy)phosphonomethylene-2,3-diaminopropionic acid benzyl ester (**12**)

Compound **4** (487.4 mg), paraformaldehyde (991.0 mg), dimethylphosphite (2.5 ml) were heated in THF (0.5 ml) at 70ºC for 20 h. The reaction mixture was diluted with 1 N $NaHCO_3$ (80 ml) and extracted with chloroform (3 × 60 ml). The combined organic phase was dried over sodium sulfate, concentrated and purified by silica column chromatography (eluting with 5 % to 7 % methanol in chloroform) to give the desired product as colorless oil, yield 336.6 mg (43 %). ^{1}H NMR ($CDCl_3$, δ in ppm) 7.39-7.33(m, 5H, -Ar*H*), 5.24(d, 1H, J = 12.17 Hz, ArCH_2-), 5.12(d, 1H, J = 12.18 Hz, ArCH_2-), 4.22(dd, 1H, J = 7.85 Hz, 5.03 Hz, -C*H*CH_2-), 3.80-3.71(m, 24H, -OCH_3), 3.43-3.13(m, 10H, -CHCH_2- and -NCH_2P-, overlapped).

N^2,N^2,N^3,N^3-Tetrakis-(bismethoxy)phosphonomethylene-2,3-diaminopropionic acid (**13**)

Compound **12** (336.6 mg) was hydrogenated under an atmosphere of hydrogen over 10 % Pd on carbon (81.7 mg) in methanol (20 ml) at room temperature for 2 h. The reaction mixture was filtered, concentrated under reduced pressure to give desired product as colorless oil, yield 252.1 mg (86 %). ^{1}H NMR ($CDCl_3$, δ in ppm) 4.29(dd, 1H, J = 8.95 Hz, 4.85 Hz, -C*H*CH_2-), 3.83-3.76(m, 24H, -OCH_3), 3.43-3.10(m, 10H, -CHCH_2- and -NCH_2P-, overlapped).

N^2-Fmoc-N^6-EDTP-2,6-diaminohexanoic acid benzyl ester (**14**)

The carboxylic acid **13** (249.8 mg), HBTU (161.3 mg), HOBt (66.0 mg) and DIEA (280 µl) were dissolved in DMF (5 ml) and Pyridine (5 ml). The mixture was stirred for 1 h. To the solution was added the amine **9** (232.0 mg) and stirred for overnight. The reaction mixture was concentrated, added chloroform (60 ml) and washed with brine (60 ml). The organic phase was dried over sodium sulfate, concentrated and purified by silica column chromatography (eluting with 5% methanol in chloroform) to give the desired product as white powder, yield 185.9 mg (38 %). ^{1}H NMR ($CDCl_3$, δ in ppm) 7.85(s, 1H, -CON*H*CH_2-), 7.76(m, 2H, -Ar*H* Fmoc), 7.62(m, 2H, -Ar*H* Fmoc), 7.41-7.31(m, 9H, -Ar*H* Fmoc and Bn, overlapped), 6.12(m, 1H, -N*H*CH-), 5.19(d, 1H,J = 12.24, -CH_2Ar Bn), 5.16(d, 1H, J = 12.23, -CH_2Ar Bn), 4.43-4.24(m, 4H, -NHC*H*- and -CH_2C*H*Ar Fmoc, overlapped), 3.99(m, 1H, -NC*H*CO-), 3.78-3.73(m, 24H, -OCH_3), 3.41-3.16(m, 12H,

-CONHCH_2-, -CHCH_2N- and -NCH_2P-, overlapped), 1.56-1.44(m, 6H, -CHCH_2CH_2CH_2-).

6-Aminohexanoic acid allyl ester mono-tosylate (**17**)

6-Aminohexanoic acid **16** (6.56 g) and *p*-toluenesulfonic acid (4.76 g) were refluxed in benzene (10 ml) and allyl alcohol (10 ml) for overnight. The reaction mixture was concentrated under reduced pressure, diluted with chloroform (100 ml) and washed 0.1 N NaOH aq (100 ml). The aqueous phase was back-extracted with chloroform (2 × 50 ml). The combined organic phase was dried over sodium sulfate and concentrated under reduced pressure. The desired product **17** was obtained as light yellow powder, yield 1.59 g (13 %) ^{1}H NMR ($CDCl_3$, δ in ppm) 7.73(d, 2H, J = 8.05 Hz, Tosylate), 7.21(d, 2H, J = 7.85 Hz, Tosylate), 5.90(m, 1H, -C*H*=CH_2), 5.29(dd, 1H, J = 1.35 Hz and 17.25 Hz, -CH=CH_2 Z), 5.22(dd, 1H, J = 1.05Hz and 10.4 Hz, -CH=CH_2 E), 4.55(d, 2H, J = 5.65 Hz, -CH_2CH=CH_2), 2.82(t, 2H, J = 7.55 Hz, εCH_2), 2.37(s, 3H, Tosylate), 2.23(t, 2H, J = 7.4 Hz, αCH_2), 1.62-1.50(m, 4H, βCH_2 and δCH_2), 1.28(dd, 2H, J = 7.85 Hz and 15.2 Hz, γCH_2).

N,N-bis(methylenephosphonic acid tetrabenzyl ester) aminohexanoic acid ally ester (**18**)

To the solution of compound **17** (1.59 g) and dibenzyl phosphonate (5 ml) in methanol (5 ml) was added 37 % formaldehyde aq (12 ml) dropwise. The reaction mixture was stirred for overnight. The mixture was diluted with 0.1 N NaOH aq (100 ml) and extracted with chloroform (3 × 70 ml). The combined organic phase was washed with brine, dried over sodium sulfate, concentrated under reduced pressure and purified by silica column chromatography (eluting with 3% methanol in dichloromethane) to give the desired product as colorless oil, yield 508.0 mg (53 %). ^{1}H NMR ($CDCl_3$, δ in ppm) 7.30(m, 20H, -Ar*H*), 5.90(m, 1H, -C*H*=CH_2), 5.32-5.21(m, 2H, -CH=CH_2), 5.04-4.94(m, 8H, ArCH_2-), 4.55(d, 2H, J = 5.65 Hz, -CH_2CH=CH_2), 3.16(d, 4H, J = 8.35 Hz, -NCH_2P-), 2.74(t, 2H, J = 7 Hz, εCH_2), 2.21(t, 2H, J = 7.55 Hz, αCH_2), 1.53(m, 2H, βCH_2), 1.37(m, 2H, δCH_2), 1.20(m, 2H, γCH_2).

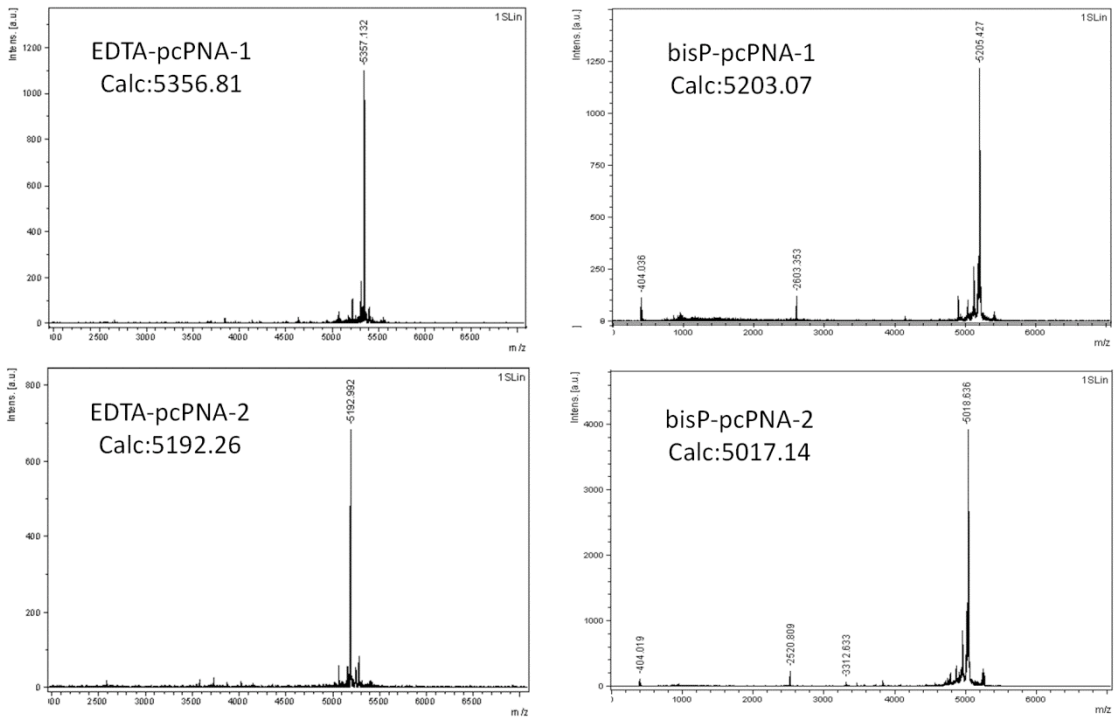

Figure S1. MS **s**pectra of EDTA-pcPNA-1, EDTA-pcPNA-2, bisP-pcPNA-1 and bisP-pcPNA-2 measured by MALDI TOF-MS.

Artificial restriction DNA cutter (ARCUT)

In ARCUT system, first two pcPNAs invade double-stranded DNA sequence-selectively at the predetermined site (**Fig. S2**). These two pcPNAs are designed to be laterally shifted to one another and two single-stranded portions are formed in both strands of the double-stranded DNA through the invasion process. Then these unpaired single-stranded sites are selectively hydrolyzed by Ce(IV)/EDTA because it preferentially hydrolyze single-stranded DNA and hardly hydrolyze double-stranded DNA. As a result, double-stranded DNA can be selectively cut at a predetermined site. ARCUT has very high site-selectivity (around 16 bp) and even whole human genome was selectively cleaved at a desired site.

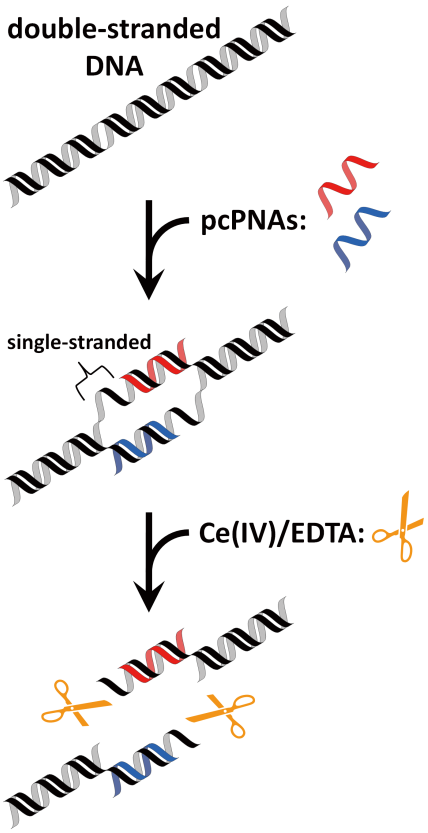

Figure S2. Schematic drawing of artificial restriction DNA cutter (ARCUT) developed by the combination of pcPNAs and Ce(IV)/EDTA to cut double-stranded DNA site-selectively.

Site-selective DNA scission by Ce(III) in the presence of several radical scavengers

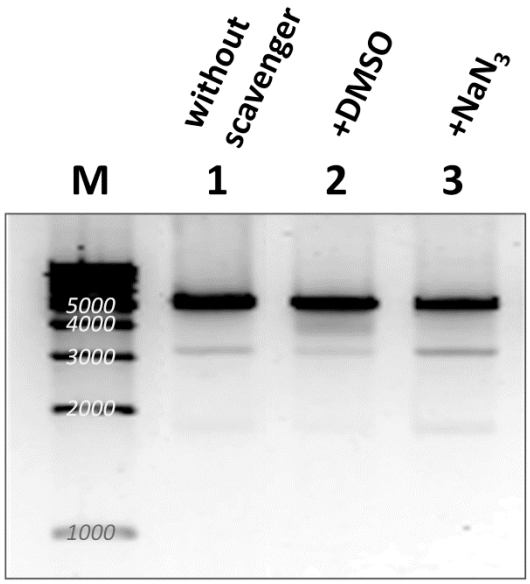

Figure S3. No retardation of radical or singlet oxygen scavengers on the site-selective DNA scission by Ce(III) and bisP-pcPNAs. Lane M, 1,000 bp ladder; Lanes 1, without scavenger; Lane 2, with 100 mM DMSO (OH radical scavenger); Lanes 3, with 10 mM NaN_3 (singlet oxygen scavenger); Reaction conditions: [linearized BFP plasmid DNA] = 4 nM, [each of bisP-pcPNAs] = 100 nM, [Hepes (pH 7.0)] = 5 mM, [NaCl] = 100 mM and [$Ce(NO_3)_3$] = 30 μM at 50^{o}C for 17 h under air.